Factor analyses differentiate clinical phenotypes of idiopathic and interferon-alpha-induced depression.

Richard Whale[a,b], Renata Fialho[a,c], Andy P Field[c], Graham Campbell[a], Jeremy Tibble[d], Neil A Harrison[a,b], Michael Rolt[a,c]

a. Immunopsychiatry Research Clinic, Sussex Education Centre, Sussex Partnership NHS Foundation Trust, Brighton, BN3 7HZ, United Kingdom

b. Department of Neuroscience, Brighton and Sussex Medical School, Brighton, BN1 9RR, United Kingdom

c. School of Psychology, University of Sussex, Brighton, BN1 9RH, United Kingdom

d. Digestive Diseases Centre, Brighton and Sussex University Hospitals NHS Trust, Brighton, BN2 5BE, United Kingdom

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Corresponding Author: Richard Whale richard.whale@brighton.ac.uk

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ABSTRACT

The discovery that prolonged administration of interferon-alpha (a pro-inflammatory cytokine) readily precipitates depressive symptoms has played a key role in development of the inflammation theory of major depressive disorder (MDD). However, it remains unclear whether the clinical phenotype of patients with inflammation-associated depression significantly overlaps with, or can be distinguished from that of patients with 'idiopathic' depression. Here we explored the Hamilton depression scale factor structure of 172 patients undergoing interferon-alpha treatment for hepatitis-C at the point of transition to a depressive episode of DSM IV defined major depression severity. The resulting factor structure was first compared with a model derived from 6 previous studies of 'idiopathic' MDD (Cole et al., 2004). This confirmatory factor analysis revealed that the factor structure of HAMD scores in our interferon-alpha treated cohort did not plausibly fit that previously described for 'idiopathic' MDD. Instead, subsequent exploratory factor analysis revealed a distinct four factor model with a novel primary factor grouping cognitive symptoms of depression and anxiety (HAMD items 1, 2, 9, 10, 11, 15). The second sleep disorder factor (items 4, 5, 6) replicated previous findings in 'idiopathic' depression. A third and unique factor grouped somatic symptoms and function (items 7, 12, 13, 14 and item 1). The final factor (also common in idiopathic depression studies), grouped gastrointestinal symptoms and weight loss (items 12 and 16). Severe depression items (3, 8, and 17) were excluded from analysis due to very low variance. At transition, interferon-alpha induced major depressive episodes therefore appears to have more associated anxiety features that covary with depressed mood than classical or 'idiopathic' MDD and a low likelihood of severe features such as suicidal ideation.

Identification of this clinical phenotype may help identify patients with an inflammatory depression etiology and support the development of more effective and personalized therapies.

1. INTRODUCTION

1.1 Sustained exposure to the pro-inflammatory cytokine interferon-alpha often precipitates a depression-like syndrome that is commonly described as appearing clinically indistinguishable from depression of other causes or 'idiopathic' depression (Raison et al., 2010). The risk of developing this syndrome increases with interferon-alpha dose and duration, and it typically remits following interferon-alpha withdrawal (Fritz-French & Tyor, 2012; Whale et al., 2015; Udina et al., 2016; Fialho et al. 2017). From meta-analysis of existing studies, at least 25% of those exposed to interferon-alpha develop a major depressive episode (MDE) within 6 months. Those with female gender, low educational level, history of major depressive disorder (MDD) or other psychiatric disorder, high baseline levels of interleukin 6 or subthreshold depressive symptoms (including psychomotor retardation and somatic symptoms) appear more vulnerable to developing this (Udina et al., 2012; Whale et al., 2015). These findings, together with now overwhelming evidence of raised inflammatory markers in at least a sub-group of patients with 'idiopathic' MDD (Haapakoski et al., 2015), have provided powerful support for the inflammatory hypothesis of depression (Lotrich, 2015; Eyre et al., 2014). However, many MDD patients show minimal or even no evidence of systemic inflammation (Miller and Raison 2016), indicating the likely existence of an etiological subgroup. Furthermore, recent evidence suggests that some classes of conventional anti-depressant medications, such as serotonin reuptake inhibitors, have less influence on inflammatory processes and poorer efficacy in inflammation- compared to non-inflammation-associated depression (Uher et al., 2014; Fialho et al., 2016).

1.2 Whether individuals with inflammation-associated depression also show a characteristic clinical phenotype is under investigation. Previous studies have demonstrated that at least in the early stages, interferon-alpha induced depression is dominated by symptoms of fatigue and reduced motivation (Capuron et al., 2002; Dowell et al., 2016). It is unclear whether these detectable differences in clinical phenotype persist with more prolonged exposure to inflammation. Capuron et al. (2009) undertook a direct comparison of HAMD item scores in interferon-alpha induced depression in a group of malignant melanoma patients (n=9), after an average of 7 weeks exposure, and an 'idiopathic' depressed patient group (n=28), finding significantly greater psychomotor retardation and weight loss scores in the interferon group, lower guilt scores and similar scores across depressed mood, anxiety and impaired activity. Reduced psychomotor speed has also been associated with raised peripheral inflammatory markers in depressed patient samples (Goldsmith et al., 2016; Krogh et al., 2014; Chamberlain et al. 2019). Identification of such clinical features, particularly if they are divergent from non-inflammation-associated or 'idiopathic' depression, may support more effective targeting of antidepressant treatments, and the development of personalized treatment strategies (Bhattacharya et al., 2016).

1.3 Here, we use confirmatory factor analysis to first identify whether the clinical phenotype of patients who developed a major depressive episode (MDE) during interferon-alpha based treatment for hepatitis-C was quantitatively different from published populations of patients with 'idiopathic' MDD. We based this analysis on

scores from the 17-item Hamilton Depression Rating Scale (HAMD), one of the most commonly used clinical tools for identifying and grading depressive symptoms. If a plausible fit between factor structures of interferon-alpha induced and 'idiopathic' depression was not observed, we aimed to use a secondary exploratory factor analysis to further characterize the specific clinical features of interferon-alpha induced depression.

2. MATERIALS AND METHODS

2.1 The study was based on a prospectively recruited cohort of 466 patients who received interferon-alpha based treatment for Hepatitis-C. The cohort consisted of consecutive referrals to the hepatology department at Brighton and Sussex University Hospital (BSUH), Brighton UK, a large specialist regional center serving a population of 0.5 million. For further details of this cohort and earlier findings, please see Whale et al., 2015. All participants had a serologically confirmed diagnosis of hepatitis-C, were aged between 18 and 65 years inclusive, and initiated PEGylated interferon-2-alpha 180 μg weekly sub-cutaneously and oral ribavirin 800-1200mg daily (depending on weight and HCV genotype) between May 2008 and April 2014. Study data from the first 24 weeks of treatment was considered, as all hepatitis-C genotypes were recommended to receive at least this period of PEGylated interferon-2-alpha treatment. Ethical approval was attained through the Brighton East National Research Ethics Committee. All participants gave written informed consent for inclusion in the study.

2.2 Participants were assessed by one of two experienced specialist hepatology nurses (MJK and AXF) at baseline and then every four weeks after initiation of interferon-alpha treatment. At each assessment point, MDE clinical threshold was explored using the Structured Clinical Interview for DSM-IV (SCID-I) (First et al., 1996), excluding criterion A12D concerning organic etiologies. Patients with a SCID-I derived baseline diagnosis of MDD or taking any antidepressant treatment prior to starting the interferon-alpha-based therapy were excluded. The 17-item HAMD was used to characterize discrete features of depression at each assessment point (Hamilton, 1960). All patients transitioning to SCID-I defined MDE were included in this study and their HAMD scores at the point of reaching the MDE threshold were entered into the subsequent factor analyses. Both specialist nurses were trained in use of the SCID-I and HAMD, including clinical case examples, to ensure between-rater reliability. Case discussions were held throughout the study including group scoring activities. All scores were reviewed by the study chief investigator (RW), a research psychiatrist.

2.3 All analyses were conducted using R 3.4.4 (R CoreTeam 2018). Normality of HAMD item scores was explored using the Shapiro Wilk test. Multivariate normality was tested using the MVN package (Korkmaz, Goksuluk, and Zararsiz 2014). Confirmatory factor analysis was conducted using the lavaan package (Rosseel 2012) and parallel analysis used the *nFactors* package (Raiche 2010). The four factor latent structure of HAMD scores described by Cole and Motivala (Cole et al., 2004) of individuals with ‘idiopathic’ DSM defined MDD was used as the MDD comparator group. This structure was itself derived from analysis of 6 previous studies with methodology reaching stringent predefined criteria, from a total of 14

studies identified between 1967 and 2002. Fit indices reported include the root mean square error of approximation (RMSEA), and Bentler's comparative fit index (CFI).

2.4 If the HAMD data for our cohort did not fit the Cole and Motivala model on confirmatory factor analysis, we next aimed to perform an exploratory factor analysis on all 17 HAMD items to define the specific factor structure of the interferon-alpha induced depression sample, methodology as supported by Schmitt (2011). This analysis was conducted using the *psych* package (Revelle 2018).

3. RESULTS

3.1 The study population consisted of 111 men and 61 women (total N=172) who transitioned to MDE within the study period. This represents a transition rate within the whole interferon-alpha exposed sample of 36.9%. Their mean age was 44.0 years, range 20 to 66, and mean time to transition to MDD following initiation of interferon-alpha was 5.6 weeks, range 4 to 24 weeks. There were no missing HAMD item values in this sample.

Table 1 shows summary statistics for each item of the HAMD. For most items scores were centered at around or below 2, the midpoint of the scale. Three items, 3 (suicide), 8 (psychomotor retardation) and 17 (loss of insight) had both a median and 75th centile of 0 indicating that at least 75% of participants responded with a 0 (out of 4). Items 2 (guilt), 9 (agitation), 12 (somatic GI), 15 (hypochondriasis) and 16 (weight loss) had a 75th centile score of 1 indicating that at least 75% of

participants responded with a 1 or lower (out of 4). At the other extreme, item 7 (work and interests) had a median of 3 and a 75th centile of 4, indicating that at least 50% of participants responded with a 3 (out of 4) and at least 25% of participants responded with the maximum score of 4. The scale total scores ranged from 7 to 37, *M* = 19.6, 95% CI [18.6, 20.5].

HAMD item	Item description (range)	n	Mean	SD	Median	Min	Max	25th	75th	Skew	Kurtosis
1	Depressed mood (0-4)	172	2.006	1.017	2.0	0	4	1	3	0.154	-0.415
2	Guilt (0-4)	172	0.767	0.847	1.0	0	3	0	1	0.686	-0.669
3	Suicide (0-4)	172	0.273	0.693	0.0	0	3	0	0	2.831	7.579
4	Insomnia initial (0-2)	172	1.366	0.709	1.5	0	2	1	2	-0.651	-0.809
5	Insomnia middle (0-2)	172	1.366	0.725	2.0	0	2	1	2	-0.675	-0.848
6	Insomnia delayed (0-2)	172	1.337	0.735	1.0	0	2	1	2	-0.528	-0.863
7	Work and interests (0-4)	172	3.023	1.037	3.0	0	4	2	4	-0.922	0.055
8	Retardation (0-4)	172	0.145	0.442	0.0	0	3	0	0	3.502	13.875
9	Agitation (0-4)	172	0.727	0.872	0.5	0	4	0	1	1.079	0.637
10	Anxiety psychic (0-4)	172	1.872	1.264	2.0	0	4	1	3	0.291	-1.084
11	Anxiety somatic (0-4)	172	1.337	0.873	1.0	0	4	1	2	0.292	-0.106
12	Somatic GI (0-2)	172	1.047	0.638	1.0	0	2	1	1	-0.039	-0.566
13	Somatic general (0-2)	172	1.605	0.557	2.0	0	2	1	2	-1.020	0.010
14	Genital symptoms (0-2)	172	1.238	0.792	1.0	0	2	1	2	-0.445	-1.283
15	Hypochondriasis (0-4)	172	0.634	0.865	0.0	0	3	0	1	1.042	-0.144
16	Weight loss (0-2)	172	0.750	0.803	1.0	0	2	0	1	0.476	-1.303
17	Insight (0-2)	172	0.058	0.258	0.0	0	2	0	0	4.709	23.751

Table 1. Hamilton Depression Rating Scale items, scored in a cohort of

patients with DSM-IV defined major depressive episode (MDE) following interferon-alpha treatment (N=172).

3.2 Confirmatory Factor Analysis

Figure 1 shows the Cole and Motivala model as fitted to our data. In this model, depression is conceptualized as a higher order psychological construct made up of four subordinate constructs of core depression (core), insomnia, anxiety and visceral symptoms. Of note, in factor analysis each of these latent constructs is revealed through analysis of the co-variance of scores on individual items of the HAMD. The fit of this conceptual model to our current data indicates how well this model of idiopathic depression describes our sample of patients with interferon-alpha induced MDE. The model significantly deviated from a perfect fit, robust χ^2 (115) = 264.59, $p < 0.001$. The robust CFI = 0.79 fell short of the recommended value of 0.9 (Bentler 1990) indicating a poor fit to the data. The robust RMSEA = 0.09 (90% CI = 0.078, 0.108) similarly confirmed this finding (MacCallum, Browne, and Sugawara 1996). In short, the four-factor latent structure of idiopathic depression described by Cole et al. (2004) is not a good description of the clinical phenotype of depression experienced by patients with DSM-IV defined MDE following interferon-alpha treatment.

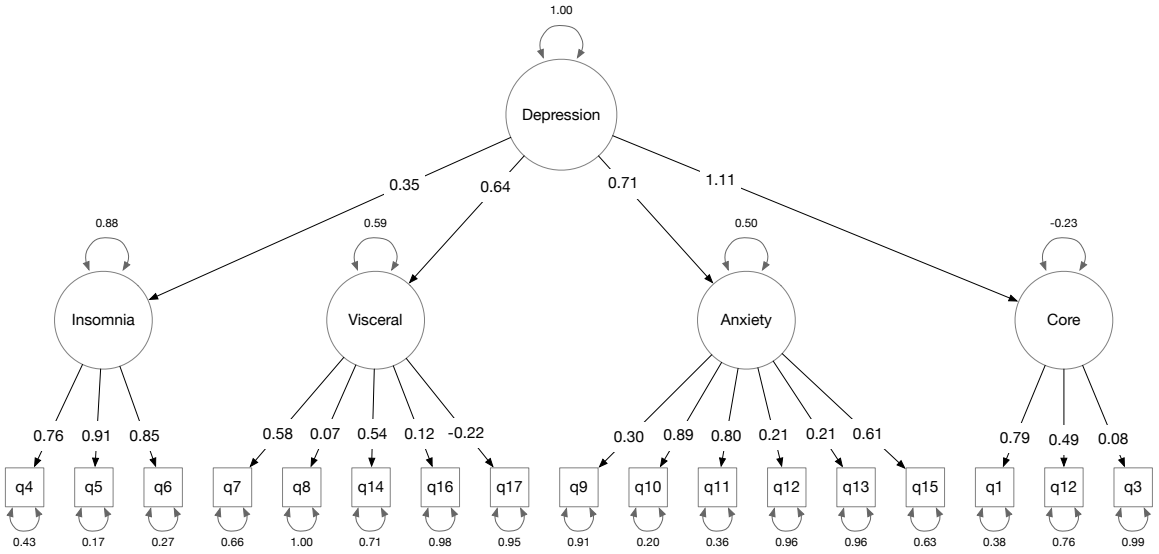

Figure 1. Confirmatory factor analysis of the 17 item Hamilton Depression Rating Scale (HAMD) in a sample of patients with interferon-alpha induced depression (items in squares), exploring goodness of fit with Cole and Motivala's rationally derived model of 'idiopathic' depression consisting of four factors (in circles): core depression (core), insomnia, anxiety and visceral. Standardized coefficients are included for each path.

3.3 Exploratory Factor Analysis on the interferon-alpha induced depression sample

Given that the Cole and Motivala model was a poor fit within our sample, we next performed an exploratory factor analysis to establish a more appropriate description of interferon-alpha induced depression. Items with virtually no variance, and therefore little discriminatory value (3, 8 and 17), were excluded. Figure 2 shows the scree plot of these data. Its point of inflexion suggests the presence of 4 underlying factors. The plot also contains the curve of corresponding

eigenvalues from randomly generated data from the same sized sample obtained from a parallel analysis (Horn, 1965). In this analysis the point at which the two curves diverge indicates the number of factors to be retained. Importantly, this again identified four discrete factors. In short, the experience of depression in patients with DSM-IV MDE following interferon-alpha treatment is best-described by clustering items on the HAMD into four factors. A factor analysis was then conducted that forced 4 factors to be extracted using the minimum residual solution (of note this approach is preferred when covariance matrices are not well behaved). The factor solution was rotated using direct oblimin. The fit of this 4-factor model was acceptable, RMSEA = 0.059 (90% CI = 0.024, 0.082).

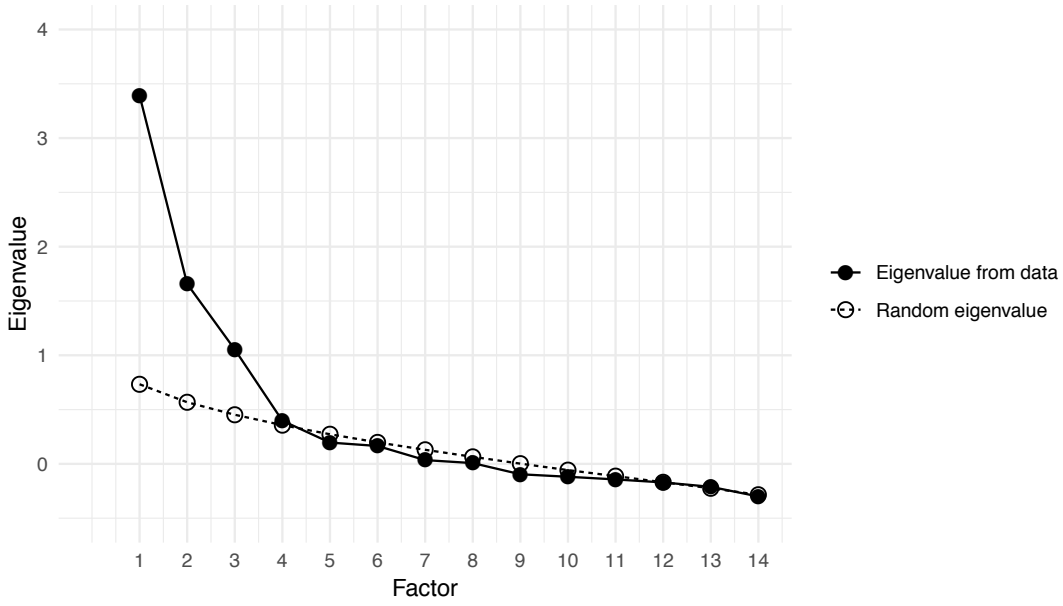

Figure 2. Scree Plot of 17 item Hamilton Depression Rating Scale scores, along with eigenvalues for random samples of the same size as the observed data (computed as per Horn's parallel analysis). Factors should be retained from the point at which the two lines deviate

Table 2 shows the pattern matrix with factor loadings greater than 0.2 in bold. Items loading highly on Factor 1 (17.2% of variance) represent cognitive symptoms of anxiety and depression: depressed mood (item 1), feelings of guilt (item 2), agitation (item 9), anxiety (items 10 and 11) and hypochondriasis (item 15). Factor 2 (15.8% of variance) represents sleep difficulties (items 4 to 6). Factor 3 (10.2% of variance) clustered physical symptoms and function: work and activities (item 7), somatic gastro-intestinal (item 12, which cross-loaded strongly with Factor 4), somatic general (item 13), genital symptoms (item14), alongside depressed mood (item 1). Factor 4 (5.1% of variance) clustered items relating to gastro-intestinal symptoms (item 12) and loss of weight (item 16). As such, the experience of depression in patients with DSM-IV defined MDE following interferon-alpha treatment is best-described in terms of symptoms clustered by anxiety/depression, sleep, somatic symptoms and daily activity, and weight loss.

HAMD	***Factor 1***	***Factor 2***	***Factor 3***	***Factor 4***
Item 1	**0.423**	0.125	**0.400**	0.013
Item 2	**0.470**	-0.093	0.120	-0.079
Item 4	-0.010	**0.786**	-0.073	0.135
Item 5	-0.032	**0.939**	-0.001	-0.037
Item 6	0.086	**0.791**	0.060	-0.071
Item 7	0.101	0.050	**0.499**	-0.082
Item 9	**0.459**	-0.211	-0.190	-0.018

Item 10	**0.873**	0.011	-0.008	0.042
Item 11	**0.782**	0.061	0.011	-0.098
Item 12	0.016	0.034	**0.382**	**0.371**
Item 13	-0.067	0.031	**0.639**	0.017
Item 14	0.002	-0.089	**0.631**	0.039
Item 15	**0.628**	0.000	-0.005	0.167
Item 16	0.024	-0.011	0.007	**0.707**

Table 2. Factor loadings from the pattern matrix of a four-factor exploratory factor analysis of Hamilton Depression Rating Scale (HAMD) items in a cohort of patients with interferon-alpha induced major depressive episode. Factor loadings greater than 0.2 are shown in bold.

4. DISCUSSION

4.1 The primary aim of this study was to quantitatively explore potential differences in the clinical phenotype of interferon-alpha induced and 'idiopathic' major depression using a factor analysis of HAMD item scores. Factor analysis provides a method for understanding how individual items cluster together and whether this clustering of clinical features matches that expected from a control sample. Direct HAMD item score comparison was not possible in the current study (as in Capuron et al. 2009, in a smaller sample). However, comparison with prior factor analyses

of ‘idiopathic’ MDD populations allowed us to demonstrate that the factor structure of our large cohort of interferon-alpha induced depression in hepatitis-C is inconsistent with that described for ‘idiopathic’ MDD. Subsequent exploratory factor analysis revealed a tighter coupling of anxiety and core depressive symptoms than is typically described for idiopathic depression.

4.1.1 The transition rate to MDE in our interferon-alpha exposed group is consistent with the existing literature (Udina et al., 2012; Udina et al., 2016). In our interferon cohort, HAMD items with very low variance related to the more severe symptoms of MDD, suicide (item 3) and loss of insight (item 17). Of note, these items (as well as psychomotor retardation which also had very low variance in our sample) have been individually excluded in previous studies of ’idiopathic’ MDD, but not as a group within a specific study (Cole et al. 2004). In previous studies of MDD, the suicide item typically clusters with ‘depressed mood’ (item 1), though this was not observed in our group. Capuron et al. (2009) similarly found low mean scores in guilt, suicide and agitation items in their interferon-alpha group. Interferon-alpha induced MDE therefore appears to have a low association with such severe depression symptoms, at least at the point of transition to depression.

4.2 Exploratory factor analysis revealed a novel 4-factor structure for interferon-alpha induced MDE. Two of these factors, (1) anxiety/depression and (3) somatic symptoms and daily activity are unique to this clinical sample in comparison with idiopathic MDD. In contrast, both the insomnia (2) and ‘anorexia’/weight loss (4) factors have previously been defined in idiopathic MDD (Marcos and Salamero 1990, Cole and Motivala 2004 and Brown et al. 1995, Gibbons et al. 1993, Pancheri et al 2002 respectively).

4.2.1 Interestingly, our factor 1 identified a co-variance in anxiety and depression symptoms in interferon-alpha induced depression that is not described in previous factor analyses of 'idiopathic' MDD. In other words, in idiopathic MDD, core mood and combined anxiety items do not typically show strong shared variance. However, this combination of core mood and anxiety symptoms does appear to be a specific feature of interferon-alpha induced MDE. Of note, items 1 and 10 (depressed mood and 'psychic' anxiety) were amongst the highest scored items in our sample. It is possible that anxiety is a more prominent feature of hepatitis-C than other interferon-alpha exposed populations (e.g. malignant melanoma) or inflamed-depressed populations (Capuron et al., 2009; Constant et al., 2005). Hepatitis-C populations also have a greater likelihood of historical substance misuse, which may complicate factor analysis of symptoms in comparison to other clinical populations, including mood and anxiety items. Our earlier description of this cohort indicated a high rate of prior intravenous drug use (94%) but any recent substance use (alcohol or illicit drug) was an exclusion factor to initiating interferon-alpha treatment (Whale et al., 2015). These issues would benefit from prospective comparison of clinical groups in future studies.

4.2.2 A somatic symptom factor has been observed in most previous HAMD factor structures (though they do not typically show consistent HAMD item configurations). In our cohort, 5 items clustered in our somatic factor (Factor 3). Only two of these items (7 and 14) were included in the Cole and Motivala 'visceral' factor. Two of our additional somatic cluster items, 1 and 7 (depressed mood and work/interests), are combined within factors with other various items in 4 previous studies (Gibbons et al. 1993, Marcos and Salamero 1990, Onega and Abraham

1997 and Pancheri et al. 2002). Thus, our factor 3 somatic items may relate more to a chronic hepatitis sample (particularly item 12, gastrointestinal somatic symptoms) rather than a mood disorder. This could be addressed in future studies comparing individuals with chronic hepatitis-C with and without MDE and those with inflammation associated MDE but not hepatitis. On a related note, use of the HAMD to assess a syndrome with important somatic aspects may miss relevant features, especially given the 0-2 point ratings of items 12, 13, 14 and 16.

4.2.3 HAMD defined psychomotor retardation was not a clear feature of interferon-alpha induced MDE in this study and was excluded from the exploratory analysis due to its very low variance. This conflicts with previous findings in smaller clinical samples (such as Capuron et al. 2009). This absence may be unique to a hepatitis-C population or to a pre-existing chronic inflammatory state. Psychomotor retardation has also been reported as an early clinical manifestation of inflammation (Capuron et al., 2002; Dowell et al., 2016) which reduces over time. Supporting this, we have previously found in this cohort that psychomotor retardation scored prior to interferon-alpha exposure is a vulnerability factor to later MDE (Whale et al. 2015). The HAMD scale may equally not be adequately sensitive to identify more experimentally identified abnormalities in motor speed, as shown by Goldsmith et al. (2016) and Krogh et al. (2014). Chamberlain et al. (2019) however found association with HAMD item 8 and raised C-reactive protein in a sample of depressed patients and controls. Further study of mood and motor/cognitive assessment in varied clinical samples at different time points would be beneficial to clarify this.

4.3 Concerning the mechanism by which interferon-alpha exposure induces this

mood syndrome, a wide biological effect has been described. Firstly interferon-alpha potently activates immune systems, as reflected particularly by increases in peripheral and brain IL6, IL1 and TNF-α (Maes, 2011; Myint et al., 2013; Hoyo-Bacerra et al., 2014). These cytokines are notably raised in some individuals with 'idiopathic' depression (Dowlati et al. 2010) and have been demonstrated to alter tetrahydrobiopterin expression, a co-factor involved in monoamine production. Endogenous interferon-alpha also mediates immune responses to pathogens and tumor cells (Hoyo-Bacerra et al., 2014). Secondly, interferon-alpha clearly induces alterations in tryptophan metabolism, stimulating indoleamine 2,3-dioxygenase activity which reduces serotonin availability, enhances quinolonic acid (enhancing glutamate neurotransmission) and reduces kynurenic acid levels, which combined have a direct neurotoxic effect and modulate monoamine release associated with mood disorder (Wohleb et al., 2016; Hepgul et al., 2016). Thirdly, interferon-alpha activates hypothalamic-pituitary-adrenal axis function, a key component of normal human physiological stress responses, and resultant glucocorticoid expression has immunomodulatory effects (Walker and Spencer 2018*).* Fourthly effects compromising neuronal plasticity and survival are also observed via interferon-alpha influence on BDNF expression and apoptotic pathways (Hoyo-Bacerra et al., 2014). Lastly, brain imaging studies of the effect of induced inflammation and depression associated inflammation have also identified potential common structures, both cortical and subcortical, which may mediate depressive disorder, and developing imaging technology may aid to identify specific pathways of phenotypic expression of differing etiologies of such disorders (Harrison, 2017). Interferon-alpha exposure therefore appears to be a possible model for inflammation associated mood disorders and induces this

illness through some common pathways identified in ‘idiopathic’ depression etiology. The factor analysis we describe suggests a differing depression phenotype in those with an interferon-alpha induced episode however.

4.3.1 Established antidepressant treatments which influence monoamine neurotransmission, have demonstrated efficacy and safety in interferon-alpha induced MDE (Schaefer et al, 2012) although the earlier neurovegetative syndrome appears less responsive to such treatment (Capuron & Miller, 2004). Anti-inflammatory medications as antidepressants appear more likely to have efficacy in inflammation associated depression (Leonard, 2014; Swardfager et al., 2016). Identification of potential clinical features of such an inflammation associated MDE is therefore of value in addition to inflammatory biomarkers to help direct the design of studies to explore optimal treatment strategies for this group.

4.4 Combining the findings discussed, an interferon-alpha induced MDE appears most likely to have prominent clinical features of cognitive symptoms of anxiety ('psychic') and depressed mood, alongside low scores in guilt, agitation, suicidal ideation and lack of insight items. The inclusion of prominent psychomotor retardation in an inflammatory depression syndrome appears likely (based on previous studies) but may be most identifiable early in the course of inflammation with specific psychomotor speed tasks. Sleep symptoms and weight loss appear to have no discriminatory value from ‘idiopathic’ MDD. Somatic features require further detailed exploration. Prospective validation of this symptom group in a clinical depressed sample using sensitive inflammatory markers and subsequent evaluation of response to anti-inflammatory agents would be of value.

4.5 A limitation of our method is that clinical depression features explored in the interferon-alpha induced group are predefined by features necessary for a SCID diagnosis of MDE, and other symptoms that may cluster around these. However, we were able to explore the relative contribution of these features. Other clinical components of an interferon-alpha induced depression syndrome or the behavioral phenotype of inflammation, particularly sub- threshold MDE features, may have been missed in this methodology. Time sensitive features of the syndrome, including the proposed early fatigue syndrome (Capuron et al, 2002) were not explored. Using time point of identification of transition to MDE may not represent peak depression severity following interferon-alpha administration and may bias HAMD item scores. Our previous findings however (Whale et al., 2015) indicate a rapid increase of HAMD score within the first 4 weeks and a plateau of severity from around 8 weeks. In this sample, mean time to transition was 5.6 weeks and by this point we would expect mean scores to be within approximately 3 HAMD points of peak, particularly considering the time requirement of 2 weeks of threshold symptoms to achieve MDE criteria. Whilst inclusion of somatic items in the HAMD scale benefits assessment of an inflammation related mood syndrome, these are measured crudely (as discussed above) and other clear mood associated features such as hopelessness and more detailed cognitive aspects leading to suicidality are neglected. The HAMD scale was initially chosen for our study to enable comparison with existing studies and for ease of clinical use but clearly only offers a superficial symptomatic assessment of a complex clinical disorder. Finding differences in depressed populations based on this scale is an important preliminary finding however. We were equally unable to explore mania-related symptoms associated with interferon exposure in this study, as reported

previously as a common consequence by Constant et al. (2005). It is possible that agitation, anxiety and insomnia items recorded by the HAMD correspond to such syndromes, although in our sample agitation and anxiety had greatest factor association with depressed mood and guilt. Of note, agitation scores were overall low in this sample. Including a mania score in a future exploratory factor analysis would be of interest. Development of a tool to more specifically identify inflammation associated depression is an important goal of such research and may be based on discriminatory HAMD items.

4.5.1 The accuracy of scoring the HAMD scale in a hepatitis-C clinic setting could also be questioned but raters were trained by a psychiatrist (RW) and scoring reliability exercises were regularly undertaken. Our findings could be by chance or related to statistical artefact, as argued in previous factor analysis studies. However, we were careful to include a large enough sample size and use robustly argued and varied statistical methods to limit this possibility. We were able to compare our findings with a rigorously developed factor model, and the numerous well conducted factor studies that led to this model including large, varied clinical samples with age and gender mix of depressed patients. Clear differences from our cohort were consistently observed. These comparator groups may have included inflammation associated MDE although this is highly unlikely to be the case for the whole sample.

4.6 To conclude, we demonstrate that our cohort of interferon-alpha induced MDD differs in HAMD factor structure from that previously reported in 'idiopathic' MDD studies and clarify further the nature of the anxiety and depression cognitive syndrome induced by interferon-alpha. Though developed in an interferon-alpha

treated cohort we argue that interferon-alpha exposure is a possible model for a more general inflammatory state reported in some patients with MDD. These findings may serve to augment clinical identification of patients with a likely inflammatory depression etiology that may be more effectively treated with novel immunotherapeutic agents.

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